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published in

From Computational Biophysics to Systems Biology (CBSB07),
Proceedings of the NIC Workshop 2007,
Ulrich H. E. Hansmann, Jan Meinke, Sandipan Mohanty,
Olav Zimmermann (Editors),
John von Neumann Institute for Computing, Jülich,
NIC Series, Vol. 36, ISBN 978-3-9810843-2-0, pp. 255-257, 2007.

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<http://www.fz-juelich.de/nic-series/volume36>

The Zinc-Finger Motif of *T.thermophilus* Ribosomal Protein S14 and the Functionality of *E.coli* Ribosomes

Georgios Papadopoulos¹, Maria A. Xaplanteri², Fotini Leontiadou³,
Theodora Choli-Papadopoulou³, and Dimitrios L. Kalpaxis²

¹ Department of Biochemistry and Biotechnology, University of Thessaly,
Ploutonos 26, 41221 Larissa, Greece
E-mail: papg@chem.auth.gr

² Laboratory of Biochemistry, School of Medicine, University of Patras,
26500 Patras, Greece

³ Laboratory of Biochemistry, School of Chemistry, Aristotle University of Thessaloniki,
54006 Thessaloniki, Greece

Protein S14 (TthS14) of the 30S ribosomal subunit from *Thermus thermophilus* contains a CXXC-X12-CXXC motif that coordinates a zinc ion. Here we report the results of experiments and molecular dynamics simulations (MDS) on the structural and functional importance of the Zn-finger and the commonly conserved cysteine 24 residue at the first position of the motif. We replaced C24 with serine and incorporated the mutants in *Escherichia coli* ribosomes. The modified ribosomes showed: a) a capability in binding tRNA at the P- and A-sites equal to that obtained with ribosomes incorporating wild-type TthS14 (20% lower compared to native *E.coli* ribosomes), b) reduced capability of the 30S subunit for association with 50S subunits after replacement of the native *E.coli* S14 by wild-type, and particularly by mutant TthS14, c) a peptidyl transferase activity in the chimeric ribosomes bearing mutant TthS14 which is unexpectedly much lower than that in ribosomes incorporating wild-type TthS14. Since the catalytic center of the ribosome is located within the 50S subunit, it seems that the perturbing effect of the S14 mutation on the catalytic center propagates by adjacent inter-subunit bridges or the P-site tRNA. This hypothesis was verified by MDS, which revealed subtle as well as large structural differences between the *E.coli* 30S-subunit head with wild type TthS14 and that with C24S mutant TthS14.

1 Introduction

Proteins in all organisms are synthesized by ribosomes. Bacterial ribosomes are assembled from two subunits (30S and 50S) with more than 50 different proteins in complex with large RNA molecules. The head of the 30S subunit contains the ribosomal protein S14 in direct contact with proteins S3 and S10 and 16S rRNA.¹ Biochemical and crystallographic studies^{2,3} indicated that S14 in *T.thermophilus* ribosomes binds a zinc ion coordinated by four cysteine residues (24, 27, 40, 43) in a CXXC-X12-CXXC motif. Zinc-fingers have been implicated in nucleic acid recognition and binding.⁴ There are, however, other bacteria, for example *E.coli*, which lack a Zn-finger motif but demonstrate rRNA binding.

Clearly, a more detailed study is needed on the significance of the Zn-motif in relation to other structural features. Here we investigated the functional importance of the S14 Zn-finger motif and its role in the assembly of the 30S subunit, in subunit association, tRNA binding and PTase activity. We performed *in vivo* experiments using *E.coli* ribosomes incorporating wild-type S14 from *T.thermophilus* (wtTthS14). We also employed targeted mutagenesis of C24 in the TthS14 Zn-finger motif to probe the role of this element in cell

growth and ribosome structure and function. Finally, we used Molecular Dynamics Simulations (MDS) to analyze the experimental results and unravel the underlying processes.

2 Materials and Methods

The biochemical methodology of this work was described elsewhere.⁶ Here we discuss the MDS details. The TthS14 structure was taken from PDB:1FJG and docked in the *E.coli* 30S-subunit head (PDB:2AVY). After solvation in a sphere with 49475 water molecules and neutralization with 343 Na ions, the structure was relaxed and equilibrated for 500 ps at 300 K (NVT). We used NAMD2⁵ and the CHARMM27 force field. Only atoms of the head of the 30S subunit (A929 to U1390 segment of 16S rRNA, proteins S3, S7, S9, S10, S13, TthS14 and S19, Mg and Zn ions) have been included in the simulation. A Cys patch with deprotonated -SH groups was applied, adopting a non-bonded model for Zn-ligand interactions. The same procedure was followed for the replacement of EcoS14 by TthS14-C24S. At the end of each equilibration, an average over the final 50 ps was taken to obtain a more representative structure, which was then used for further analysis. The $|\vec{r}_{gc}^{mut} - \vec{r}_{gc}^{wt}|$ distances of the geometrical centers for all residues were calculated for the final structures.

3 Results and Discussion

Table 1 summarizes the results of TthS14 incorporation in *E.coli* ribosomes and 70S reconstruction experiments. The results suggest an S14 role in the association of ribosomal subunits. Disturbances in the S14 structure apparently induce changes in other components of the 30S subunit head, for example residues of the S13 protein and 16S rRNA, which are directly implicated in subunit association.⁷ Our MDS results showed significant shifts (Fig. 1) for several 16S rRNA groups in inter-subunit bridges. We found that the Zn ion moves towards two oxygen residues (O2 and O2') of U1202, and away from C24. This behavior can be expected only for TthS14-C24S. In the wtTthS14 case, it is rather striking and it may be attributed to the non-bonded model for the Zn^{2+} -ligand interactions, adopted in this study. For both species, the Zn ion stays close to C27, C40, and C43.

Assembled protein	Degree of incorporation (%)	70S ribosomes (%)
EcoS14	100.0	81.6 \pm 5.1
wtTthS14	88.5	75.0 \pm 3.2
TthS14-C24S	57.4	66.0 \pm 4.5

Table 1. Degree of incorporation of TthS14 and TthS14-C24S proteins into *E.coli* ribosomes and ability of the resulting ribosomes to form 70S complexes.

The tRNA affinity was not affected by the C24S mutation, as confirmed by the MDS. All 16S rRNA residues implicated previously^{1,8} in tRNA binding to the P-site, except m²G966, relaxed to almost identical geometries. Regarding PTase activity our experiments show an eight times smaller value for ribosomes with S14 C24S. Provided that there are not direct contacts of S14 to the catalytic center, it is tempting to speculate, that the observed

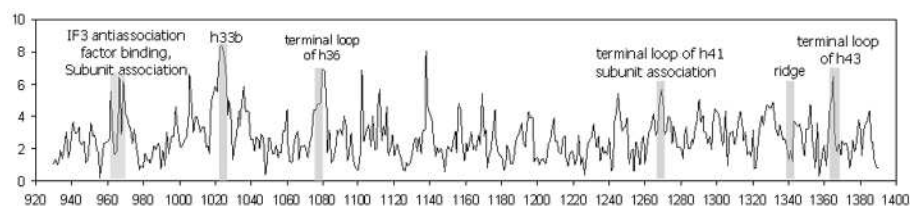


Figure 1. Displacements of 16S rRNA groups after mutation of TthS14 C24 into S24. Regions implicated in subunit association and tRNA binding are in grey.

by MDS conformational changes initiated by the C24S mutation are transmitted through adjacently located intersubunit bridges (bridge B1a or B1b) or through the P-site bound tRNA and cause a less reactive reorientation in the catalytic center.

Acknowledgments

This work was supported by Grant 99ED-605 from the GSRT, Greece, and the European Social Fund. We thank C. M. McCallum for providing the Cys-patch.

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